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ENERGETIC MATERIALS EFFECTS ON ESSENTIAL SOIL PROCESSES: DECOMPOSITION OF ORCHARD GRASS (*DACTYLIS GLOMERATA*) LITTER IN SOIL CONTAMINATED WITH ENERGETIC MATERIALS

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We investigated the effects of individual nitrogen-based energetic materials (EMs) 2,4-dinitrotoluene (2,4-DNT), 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), nitroglycerin (NG), and 2,4,6,8,10,12-hexanitrohexaazaisowurtzitane (CL-20) on decomposition, an essential soil process, utilizing Orchard grass (*Dactylis glomerata*) straw in Sassafras sandy loam (SSL) soil. Three pieces of 5-cm long internodular sections of straw were used to form a straw cluster. Pre-weighed straw clusters were placed in the soil in each 900 mL volume test container containing approximately 200 g of loosely packed EM-amended soil or carrier (acetone) control soil. After 1, 2, 3, 4, 6, and 8 months of exposure, one straw cluster was harvested from a set of randomly selected replicate containers from within each treatment to quantify the decomposition rates. The exposure concentrations of each EM in soil were analytically determined soils using the U.S. Environmental Protection Agency Method 8330A. The results showed that soil contamination with 2,4-DNT or NG can inhibit litter decomposition rates based on the EC_{50} values of 1122 mg/kg and 860 mg/kg, respectively. Exposure to 2-ADNT, 4-ADNT or CL-20 did not significantly affect litter decomposition in SSL soil at ≥10000 mg/kg. These ecotoxicological benchmarks can help identify concentrations of contaminant EM in soil that present an acceptable ecological risk for biologically-mediated processes in soil.

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PREFACE

The work described in this report was authorized under project no. SERDP ER-1416 and SERDP ER-1254. The work was started in July 2002 and completed in December 2012.

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ENERGETIC MATERIALS EFFECTS ON ESSENTIAL SOIL PROCESSES: DECOMPOSITION OF ORCHARD GRASS (*DACTYLIS GLOMERATA*) LITTER IN SOIL CONTAMINATED WITH ENERGETIC MATERIALS

1. INTRODUCTION

Increased demand for military training and testing resources in recent years has resulted in increased levels of energetic materials (EMs) in soils and has raised concern regarding their environmental impacts at testing and training ranges. The extent of land that has been contaminated exceeds 15 million acres by some accounts (U.S. Government Accounting Office [USGAO], 2003). Among the common energetic residues found in range soil are 2,4-dinitrotoluene (2,4-DNT), and nitroglycerin (NG). 2,4-DNT does not mineralize once exposed to the environment either aerobically or anaerobically, but it can be environmentally transformed to a variety of nitroaromatic species (Jenkins, 2007; Monteil-Rivera et al., 2009). Partially reduced degradation products of 2,4,6-trinitrotoluene (TNT) and DNTs, 2-amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT) frequently co-occur in soil contaminated with nitroaromatic EMs (Kuperman et al., 2009a). NG can be released into the environment from the nitrocellulose matrix of solid propellants used in rockets and artillery ammunitions. NG is mobile in soil due to its moderate aqueous solubility of 1.8 g/L at 20 °C (Verscheuren, 1983; Pal and Ryon, 1986), and low partition coefficient values such as log K_{ow} of 1.62 (Sunahara et al., 2009) and log K_{oc} of 2.77 (Spanggord et al., 1980). Environmental assessments conducted at 23 military firing ranges in the United States and Canada identified NG as a soil contaminant at antitank rocket ranges with concentrations in soil as high as 4700 mg/kg (Jenkins et al., 2006).

An emerging polynitramine energetic material 2,4,6,8,10,12-hexanitrohexaazaisowurtzitane (CL-20) is being considered as a potential replacement for existing cyclic nitramine explosives hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX). Understanding of potential ecological impacts of its release into the environment is necessary prior to large-scale production of CL-20. It contains multiple electron-withdrawing N-NO_2 functional groups, causing the explosive to resist electrophilic attack by oxygenases under aerobic soil conditions resulting in slow and incomplete mineralization (Hawari, 1999; Hawari et al., 2004; Monteil-Rivera, 2009).

Notwithstanding the persistence of these EMs in soil, their effects on key soil processes that are important to the regulation, flow, and internal cycling of carbon and nutrients in ecosystems have not been sufficiently investigated (Kuperman et al., 2009a). As a result, scientifically defensible screening values for biologically-mediated processes in soil, which could be used in ecological risk assessment (ERA) at EM-contaminated sites, are not currently available for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20.

Litter decomposition is an essential soil process, and one of the most integrating processes within the soil ecosystem because it involves complex interactions of soil microbial and faunal activity with the soil chemical environment (Wentsel et al., 2003; Kuperman et al., 2002). Any disturbance that alters organic matter decomposition can result in nutrient losses and a decline in soil fertility. Therefore, an assessment of how soil contamination with EMs may alter rates of organic matter decomposition in soil is critical to understanding potential EM

impact on overall ecosystem function. Inclusion of assessment of EM effects on litter decomposition can also generate data that provide information on the level of reliability, practicality, and relative sensitivity of microbial assessment endpoints, if used in conjunction with ecological soil screening levels (Eco-SSLs; http://www.epa.gov/ecotox/ecossl/; last accessed March 2013) within the framework of the screening level ERA (SLERA) at EM-contaminated sites. To fill data gaps, we conducted definitive studies to develop new ecotoxicological data, which can aid site managers to make knowledge-based decisions to secure sustainable use of testing and training installations. These studies were performed as part of Strategic Environmental Research and Development Program (SERDP)-funded projects (SERDP ER-1254 and SERDP ER-1416).

2. MATERIALS AND METHODS

2.1 Soil Collection and Characterization

Several collections of Sassafras sandy loam (SSL), a fine-loamy, siliceous, semiactive, mesic Typic Hapludult soil, containing indigenous microbial and invertebrate communities, were used throughout studies within related SERDP projects that investigated the environmental fate and ecotoxicological effects of EMs. The qualitative relative bioavailability score for organic chemicals in natural soils were considered very high for SSL according to the Eco-SSL criteria (USEPA, 2005). Characteristics of two of these collections of SSL soil used in the present studies are summarized in Table 1. The collection designated SSL2003 was used for studies with CL-20 (SERDP ER-1254; Kuperman et al., 2006a). SSL2007e was used to assess the effects of 2,4-DNT, 2-ADNT, 4-ADNT, and NG (SERDP ER-1416; Sunahara, 2012).

Table 1. Physical and Chemical Characteristics of SSL Soil Collections Used in Toxicity Testing

Soil Parameter	SSL2003	SSL2007e
Sand, %	58	62
Silt, %	28	25
Clay, %	14	13
Texture	Sandy loam	Sandy loam
Cation exchange capacity (CEC), cmol/kg	9.8	7.8
Organic matter, %	2.6	2.2
pH	5.1	5.0

During soil collection, the root zone of the upper soil layer was retained to ensure sufficient abundance of the indigenous soil organisms. Fresh SSL2003 soil was collected from an open grassland field in the coastal plain on the property of the U.S. Army Aberdeen Proving Ground (APG), Harford County, Maryland, in May 2003. Fresh SSL2007e soil was collected from the same location in November 2007. Soil was gently passed through a 5-mm sieve to remove large debris and regularize distribution of soil organisms, then stored in covered plastic containers overnight to preserve the initial field moisture level.

For each of the respective studies, CL-20 (SERDP ER-1254; Kuperman et al., 2006a) and 2,4-DNT, 2-ADNT, 4-ADNT, and NG (SERDP ER-1416; Sunahara, 2012), additional SSL was collected several days before each study commenced for preparation of EM soil concentrates. In order to prepare EM concentrates, prior to amendment of this soil with EM soil batches were treated by prolonged heating (three days after constant mass was achieved) at 80 °C. This was necessary in order to minimize potential introduction of additional organisms, otherwise present in these batches of soil, to the overall biological activity (combined microbial and micro-invertebrate communities) in the final soil treatments of EMs. The heat-treated soil batches were sieved through a 2-mm sieve, and subsequently used to prepare EM soil concentrates for the respective studies with 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20.

2.2 Chemicals and Reagents

EMs used in the studies included nitroaromatics 2,4-DNT [Chemical Abstracts Service (CAS) no. 121-14-2; purity: 97%], 2-ADNT (CAS no. 35572-78-2; purity 99%), 4-ADNT (CAS no. 19406-51-0; purity 99%), and NG (CAS no. 55-63-0; purity: 99%), as well as the nitramine CL-20 (CAS no. 135285-90-4; ε-isomer, purity 99.3%). CL-20 was obtained from ATK Thiokol Propulsion (Ogden, UT, USA). The remaining EMs were obtained from Defense Research and Development Canada-Valcartier (Quebec City, Quebec, Canada). High-performance liquid chromatography (HPLC)-grade acetone (CAS no. 67-64-1) was used to prepare individual EM solutions prior to soil amendments (EM soil concentrates). Acetonitrile (ACN) (CAS no. 75-05-8; HPLC grade), methanol (CAS no. 67-56-1; chromatography grade; purity, 99.9%), and calcium chloride ($CaCl_2$) (CAS no. 10043-52-4; reagent grade) were used for the soil extractions and in analytical determinations by HPLC. Certified standards of EMs (AccuStandard, Inc., New Haven, CT) were used in HPLC determinations. American Society for Testing and Materials (ASTM) International (West Conshohocken, PA) Type I water (18 MΩ cm at 25 °C; ASTM, 2004) was used throughout the toxicity studies. It was obtained using Milli-RO 10 Plus followed by Milli-Q PF Plus systems (Millipore, Bedford, MA). The same grade of water was used throughout the analytical determinations. Glassware was washed with phosphate-free detergent and sequentially rinsed with tap water, ASTM Type II water (>5 MΩ cm at 25 °C), analytical reagent-grade nitric acid 1% (v/v), and ASTM Type I water.

2.3 Soil Amendment Procedures

Studies were performed separately and independently for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 to determine the effects of each EM on decomposition of Orchard grass (*Dactylis glomerata*) straw. Soil concentrates of each EM were required to uniformly amend fresh field-moist SSL soil during preparation of nominal target treatment concentrations in order to avoid harming soil organisms by exposure to carrier solvent (acetone).

Individual soil concentrates of 2,4-DNT, 2-ADNT, 4-ADNT, and NG were prepared in three steps. In the first step, concentrates of 1000 (Concentrate I) or 10,000 (Concentrate II) mg/kg were prepared by adding 0.1605 or 1.5002 g of crystalline EM to 60 mL of acetone (in three consecutive additions of 20 mL aliquots), then pipetting the respective EM/acetone mixtures onto 159.8 or 148.5 g batches of heat-treated SSL soil. A separate EM concentrate (Concentrate III) was prepared for the target 10,000 mg/kg treatment by adding 14.0045 g of crystalline EM to 60 mL of acetone, and then pipetting the EM/acetone mixture onto 126 g of heat-treated SSL soil. The acetone was allowed to volatilize overnight in a

fume hood in darkness. In order to fully-distribute the EM within respective batches, each amended heat-treated SSL soil batch was then mixed for 18 h using a three-dimensional rotary soil mixer. In the second step, an intermediate EM concentrate (Concentrate IV) was prepared for the target 10 mg/kg treatment by adding 14 g of EM Concentrate I to 126 g of heat-treated SSL soil, then mixing the components for 18 h using a three-dimensional rotary soil mixer. The final target EM treatments were prepared in the third step. Target 10 mg/kg treatment was prepared by adding 140 g of Concentrate IV to 1260 g of fresh SSL2007e soil. Target 100 or 1000 mg/kg treatments were prepared by adding 140 g of EM Concentrates I or II, respectively to 1260 g of fresh SSL2007e soil. Target EM treatment of 10,000 mg/kg was prepared by adding 140 g of Concentrate III to 1260 g of fresh SSL2007e soil. All treatments were prepared one day after collecting SSL2007e soil in the field by individually combining and gently mixing EM soil concentrates with clean SSL2007e soil in separate plastic bags. The carrier (acetone) control treatment was prepared by adding 140 g of Concentrate 0 to 1260 g of fresh SSL2007e soil.

The CL-20 soil concentrate was prepared by dissolving appropriate amounts of CL-20 in 15 g of acetone and pipetting onto measured portions of heat-treated SSL soil. This approach was used to prepare nominal CL-20 treatments of 100, 500, 1000, and 2500 mg/kg. Nominal CL-20 treatments of 5000, 7500, and 10,000 mg/kg were prepared by directly mixing appropriate amounts of CL-20 with heat-treated SSL soil because large quantities of CL-20 required for preparation of these treatments could not be dissolved in 15 g of acetone. Acetone was added to these three treatments in the same amount (15 g) to maintain the uniformity of treatments throughout all exposure concentrations. The acetone was allowed to volatilize in a chemical fume hood overnight in the dark. In order to fully-distribute the EM within respective batches, each amended soil batch was then mixed for 18 h using a three-dimensional rotary soil mixer. All treatments were prepared one day after collecting SSL2003 soil in the field by individually combining and gently mixing CL-20 soil concentrates with clean SSL2003 soil in separate plastic bags. The carrier control treatment was amended with acetone-treated SSL soil only. The final nominal CL-20 treatment concentrations prepared for definitive test included 0 (carrier control), 100, 500, 1000, 2500, 5000, 7500, and 10,000 mg/kg.

The above approach ensured that the amount of fresh SSL2007e or SSL2003 soil, respectively, containing indigenous organisms remained constant throughout the range of treatments. The field soil moisture level of 14% dry soil mass was maintained for the duration of the study by weekly additions of ASTM Type I water. Soil samples were collected from each EM treatment and carrier controls for analytical determination of EM concentrations.

2.4 Extraction of EMs from Soil

Concentrations of EMs in all control and treated soils were analytically determined, in triplicate, at the beginning of each definitive test using ACN extraction and U.S. EPA Method 8330A (USEPA, 2007). Soil dry-fraction (dry weight/wet weight) was determined in triplicate from subsamples of each treatment concentration. For extraction, triplicate 2 g treatment or control samples were collected from each soil treatment batch and placed into respective 50 mL polypropylene centrifuge tubes, and 10 mL of ACN was added to each tube. Internal standards were added (100 µL) to each tube to evaluate the extraction efficiency. Internal standards were 1,3-dinitrobenzene (1,3-DNB) for 2,4-DNT, 2-ADNT, and 4-ADNT; and HMX for NG. Soil extraction was repeated if internal standard recovery was less than 90%. Samples were vortexed with ACN for 1 min, then sonicated in darkness for 18 h at 20 °C. After

the sonicated samples had settled for 1 h at room temperature, 5 mL of each supernatant was transferred into glass tubes that contained 5 mL of $CaCl_2$ solution (5 g/L) as a flocculent. Supernatant was filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe cartridge, and 1 mL of each filtered solution was transferred into an HPLC vial. Soil extracts were analyzed and quantified by HPLC.

2.5 Analytical Determinations of EMs in Soil

Concentrations of 2,4-DNT, 2-ADNT, 4-ADNT, and NG in the soil extracts were quantified using a Waters Corporation (Milford, MA) HPLC system composed of a Model 600 pump, a Model 717 Plus injector, a Model 2996 photodiode-array, and a temperature control module. Calibration curves were generated before each HPLC run using certified standards (AccuStandard, New Haven, CT or Cerilliant, Round Rock, TX) of each EM, in a range of concentrations appropriate for each set of determinations. The limits of detection were 0.01, 0.005, 0.005, and 0.05 mg/L for 2,4-DNT, 2-ADNT, 4-ADNT, and NG, respectively, corresponding to 0.1, 0.05, 0.05, and 0.5 mg/kg (dry soil mass). Concentrations of CL-20 in the soil extracts were determined using a high-performance liquid chromatography-ultraviolet (HPLC-UV) system, which consisted of an Agilent Technologies (Santa Clara, CA) 1100 HPLC Series equipped with a Sigma-Aldrich Co., LLC (St. Louis, MO) Supelcosil LC-CN column (25 cm x 4.6 mm x 5 µm), employing an isocratic 70:30 methanol:water mobile phase with a flow rate of 1.0 mL/min and a 50 µL injection volume. The autosampler was set at 10 °C. Blanks and standards were placed among samples having unknown concentration in order to maintain quality assurance of the samples. Detection of CL-20 was accomplished using a diode array detector set at 230 nm (λ_{max}) wavelength. A primary stock solution was prepared at 10000 mg/L CL-20 in ACN. Intermediate stock solutions of 50, 20, 2, 0.5, and 0.1 mg/L CL-20 in ACN were then prepared from the primary stock solution. Calibration standards were made from the intermediate stock solutions with acidified water (sodium bisulfate) solution (50:50) to yield standards of 25, 10, 1, 0.25, and 0.05 mg/L CL-20 in acidified ACN. Calibration curves were created (r^2>0.99999) with an instrument limit of detection (LOD) of 0.01 mg/L (S/N=3). Over five months, the reproducibility of the slope was determined to be 149.0±5.0 with a % relative standard deviation (RSD) of 3.4 (n=14). The lowest concentration of CL-20 that could be quantified in freshly amended SSL soil was in the nominal treatment of 0.08 mg/kg, and was 0.098 mg/kg. The lowest quantified concentration of CL-20 weathered and aged in SSL soil was in the nominal treatment of 0.1 mg/kg, corresponding to 0.06 mg/kg. All chemical concentrations in soil were expressed on dry mass basis. Nominal and analytically determined (measured) concentrations used in the definitive Litter Decomposition Assays are shown in Tables 2–6.

2.6 Litter Decomposition Assay

Litter decomposition rates were quantified using Orchard grass (*D. glomerata*) straw collected in a grassland on the property of APG, in May 2003 for studies with CL-20, and in May 2007 for studies with the remaining EMs. Orchard grass straw was dried at 60 °C until constant mass was achieved, then cut into 5-cm long internodular sections. Three pieces of these straw sections were used to form a straw cluster. The mass of each cluster was recorded and an aluminum tag with an identification number was attached to each cluster with a nylon string. Six pre-weighed straw clusters of approximately 0.2 g each were placed in the soil in each test container (glass jars 900 mL volume, 90 mm diameter) containing approximately 200 g of loosely packed EM-amended soil or carrier control soil. All containers were placed randomly in

an environment-controlled incubator under a 16 h light–8 h dark photoperiod cycle with a mean photosynthetically active radiation (PAR) light intensity of 12.8 ± 0.7 µM m^{-2}/s (985 ± 52 lux), 86% relative humidity, and mean temperature of 22±1 °C. ASTM Type I water was added each week in order to maintain the initial soil moisture level.

One cluster of Orchard grass straw was harvested from a set of randomly selected replicate containers (n=3 in studies with CL-20; n=4 in studies with remaining EMs) from within each treatment after 1, 2, 3, 4, 6, and 8 months of exposure until approximately 80% mass loss was recorded in the treatment with the greatest decomposition rate. Harvested clusters of Orchard grass straw were gently rinsed with ASTM Type I water to remove soil debris, then oven-dried at 60 °C until constant mass was achieved (approximately 24 h).

2.7 Data Analyses

Annual decomposition rate constants (*k*) for litter residues, and corresponding standard errors (SE) and regression coefficients (r^2) were determined using the single exponential decay model $m_t/m_0 = e^{-kt}$, where m_t/m_0 = fraction mass remaining at time t, t = time elapsed in years, and *k* = the annual decomposition constant (Kuperman, 1999). The model was fit to the data by least squares regression of the natural logarithm of mean percent mass remaining over time. Decomposition rate data were analyzed using regression models selected among those described in Environment Canada Guidance Document (EC, 2005). During the model selection process, compliance with the normality assumptions and homoscedasticity of the residuals were determined by examining the stem-and-leaf graphs and histograms of the residuals. Best fit for the decomposition rate data was the exponential model

$$Y = a \times e(([\log(1-p)] \div \mathrm{ECp}) \times \mathrm{C}) + b$$

where *Y* = dependent variable for a measurement endpoint (i.e., annual decomposition rate constants); *a* = the y-axis intercept (i.e., the control response); *e* = the exponent of the base of the natural logarithm; *p* = desired value for '*p*' effect (e.g., 0.50 for a 50% decrease from the control response; EC_{50}); C = the exposure concentration in test soil; ECp = estimate of effect concentration for a specified percent effect; and *b* = a scale parameter that defines the shape of the equation. The best fit was evident when the regression lines generated by the models were closest to the data points, the regression coefficients for point estimates were the greatest, the residuals were homoscedastic (i.e., had most random scattering), and the means, standard errors, and variances of the residuals were the smallest. The 95% confidence intervals (CI) associated with the point estimates were determined.

Analysis of variance (ANOVA) was used to determine the bounded (when possible) no-observed-effect concentration (NOEC) and the lowest-observed-effect-concentration (LOEC) values for the effects of EMs on litter mass loss or decomposition rates. Mean separations were done using Fisher's least-significant difference (FLSD) pairwise comparison tests. All statistical analyses were done using analytically determined EM concentrations. A significance level of $p \leq 0.05$ was accepted for all statistical tests. Statistical analyses were performed using SYSTAT 11 (Systat Software, Inc., Chicago, IL).

3. RESULTS

3.1 Analytical Determinations of EMs in Soil

Concentrations of 2,4-DNT were analytically determined in triplicate for each freshly amended treatment of SSL2007e soil to establish a baseline of initial concentrations for litter decomposition study. The initial concentrations of 2,4-DNT in the two lowest treatments were 38% and 62% of nominal 10 and 100 mg/kg treatments, respectively. These comparatively low recovery rates suggest rapid biotransformation processes in freshly amended soils. The initial analytically determined 2,4-DNT concentrations that yielded recovery percentages 127% and 93% were more consistent with the respective target concentrations of 1000 and 10,000 mg/kg, respectively (Table 2). Concentrations of 2,4-DNT in each treatment were analytically determined on each straw harvest date during the eight-month study. Percent recovery declined steadily over time in 10, 100, and 1000 mg/kg nominal treatments, but at a greater rate in the lowest two nominal treatments (Table 2). Concentrations of 2,4-DNT remained relatively stable in the greatest nominal treatment of 10,000 mg/kg, providing indirect evidence of inhibition of microbial activity at this concentration.

Table 2. Nominal and Analytically Determined Concentrations of 2,4-DNT in Sassafras Sandy Loam Soil During the Eight-month Litter Decomposition Study with *Dactylis glomerata*

Nominal concentration	10		100		1000		10000	
	Mean (SD) mg/kg	Recovery (%)	Mean (SD) mg/kg	Recovery (%)	Mean (SD) mg/kg	Recovery (%)	Mean (SD) mg/kg	Recovery (%)
Measured Initial	3.8 (0.4)	38†	62 (3.2)	62†	1274 (19)	127†	9343 (501)	93†
1 Month	2.1 (0.02)	55	34 (1.6)	56	887 (14)	70	13467 (676)	144
2 Months	2.4 (0.4)	63	24 (0.8)	40	1024 (49)	80	12637 (569)	135
3 Months	1.1 (0.04)	30	16 (1.1)	27	843 (5)	66	10145 (899)	109
4 Months	1.4 (0.1)	37	12 (0.2)	20	853 (35)	67	12585 (1216)	135
6 Months	0.6 (0.1)	16	7 (0.4)	12	614 (54)	48	10893 (625)	117
8 Months	0.6 (0.01)	16	6 (0.6)	9	740 (73)	58	10958 (637)	117

Notes: †Percent initial recovery of 2,4-DNT from freshly amended SSL soil compared with nominal target concentration; subsequent recovery values show percent change from the initial analytically determined concentration in freshly amended soil during the 8-month study. Measured concentrations are based on USEPA Method 8330A; Values are means (n=3) and Standard Deviations (SD).

Concentrations of 2-ADNT were analytically determined in triplicate for each freshly amended treatment of SSL2007e soil to establish a baseline of initial concentrations for litter decomposition study. The initial analytically determined concentrations of 2-ADNT were consistent with the respective target concentrations (Table 3). Concentrations of 2-ADNT in each treatment were analytically determined on each straw harvest date during the eight-month study. Percent recovery declined steadily over time in 10, 100, and 1000 mg/kg nominal treatments, but at a greater rate in the lowest two nominal treatments (Table 3). Concentrations of 2-ADNT remained relatively stable in the greatest nominal treatment of 10,000 mg/kg, providing indirect evidence of inhibition of microbial activity at this concentration.

Table 3. Nominal and Analytically Determined Concentrations of 2-ADNT in Sassafras Sandy Loam Soil During the Eight-month Litter Decomposition Study with *Dactylis glomerata*

Nominal concentration	10		100		1000		10000	
	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)
	mg/kg		mg/kg		mg/kg		mg/kg	
Measured Initial	10 (0.4)	100[†]	117 (5)	117[†]	1200 (55)	120[†]	10000 (500)	100[†]
1 Month	4.9 (0.04)	47	60 (4)	51	1297 (89)	109	8980 (423)	90
2 Months	4.34 (0.2)	41	51 (4)	44	1113 (107)	94	10142 (925)	101
3 Months	2.8 (0.2)	27	46 (3)	39	1079 (63)	91	10571 (517)	106
4 Months	2.1 (0.1)	20	33 (0.3)	28	804 (8)	68	8447 (40)	84
6 Months	1.4 (0.1)	13	22 (1)	18	938 (86)	79	8738 (213)	87
8 Months	1.2 (0.02)	12	18 (1)	15	1025 (26)	86	9815 (615)	98

Note: [†]Percent initial recovery of 2-ADNT from freshly amended SSL soil compared with nominal target concentration; subsequent recovery values show percent change from the initial analytically determined concentration in freshly amended soil during the 8-month study. Measured concentrations are based on USEPA Method 8330A; Values are means (n=3) and standard deviations (SD).

Concentrations of 4-ADNT were analytically determined in triplicate for each freshly amended treatment of SSL2007e soil to establish a baseline of initial concentrations for litter decomposition study. The initial analytically determined concentrations of 4-ADNT were generally consistent with the respective target concentrations (Table 4). Concentrations of 4-ADNT in each treatment were analytically determined on each straw harvest date during the eight-month study. Percent recovery declined steadily over time in all nominal treatments, but at a greater rate in the lowest nominal treatment of 100 mg/kg, providing an indirect evidence of lower inhibition of microbial activity at this concentration compared with inhibition in greater nominal treatments (Table 4).

Table 4. Nominal and Analytically Determined Concentrations of 4-ADNT in Sassafras Sandy Loam Soil During the Eight-month Litter Decomposition Study with *Dactylis glomerata*

Nominal concentration	100		1000		5000		10000	
	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)
	mg/kg		mg/kg		mg/kg		mg/kg	
Measured Initial	113 (9)	113†	1380 (34)	138†	5880 (561)	117†	12560 (606)	126†
1 Month	51 (0.4)	45	765 (73)	56	4216 (44)	72	7445 (451)	59
2 Months	45 (1)	40	1019 (3)	74	3903 (183)	66	7943 (1183)	63
3 Months	34 (0.3)	30	751 (58)	55	4049 (16)	69	8115 (98)	65
4 Months	29 (1)	26	703 (34)	51	3759 (332)	64	8133 (755)	65
6 Months	22 (1.5)	19	652 (53)	47	3909 (153)	66	7368 (238)	59
8 Months	14 (0.2)	12	675 (21)	49	3985 (107)	68	7700 (405)	61

Note: †Percent initial recovery of 4-ADNT from freshly amended SSL soil compared with nominal target concentration; subsequent recovery values show percent change from the initial analytically determined concentration in freshly amended soil during the 8-month study. Measured concentrations are based on USEPA Method 8330A; Values are means (n=3) and standard deviations (SD).

Concentrations of NG were analytically determined in triplicate for each freshly amended treatment of SSL2007e soil to establish a baseline of initial concentrations for litter decomposition study. The initial concentration of NG in the lowest treatment was 29% of the nominal 100 mg/kg target value. This low recovery rate suggests rapid biotransformation processes in freshly amended soil. The remaining analytically determined initial treatment concentrations of NG were generally consistent with the respective nominal target concentrations of 1000, 5000, and 10,000 mg/kg (Table 5). Concentrations of NG in each treatment were analytically determined on each straw harvest date during the eight-month study. Percent recovery declined steadily over time in all treatments, but at a greater rate in the lowest two nominal treatments of 100 and 1000 mg/kg, providing indirect evidence of inhibition of microbial activity in the nominal 5000 and 10,000 mg/kg treatments (Table 5).

Concentrations of CL-20 in SSL2003 soil treatments remained relatively stable during the 8-month study, and ranged on average from 88−102% of the initial concentrations in freshly amended soil (Table 6).

Table 5. Nominal and Analytically Determined Concentrations of NG in Sassafras Sandy Loam Soil During the Eight-month Litter Decomposition Study with *Dactylis glomerata*

Nominal concentration	100		1000		5000		10,000	
	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)	Mean (SD)	Recovery (%)
	mg/kg		mg/kg		mg/kg		mg/kg	
Measured Initial	29 (1)	29[†]	950 (48)	95[†]	5000 (209)	100[†]	10000 (46)	100[†]
1 Month	3.3 (0.1)	11	620 (33)	66	4639 (216)	94	8797 (538)	88
2 Months	1.1 (0.01)	4	481 (12)	51	3879 (84)	78	8203 (388)	82
3 Months	0.6 (0.2)	2	340 (7)	36	3642 (254)	74	7525 (525)	75
4 Months	0	0	350 (2)	37	4169 (81)	84	8038 (352)	80
6 Months	0.7 (0.1)	2	273 (8)	29	3663 (236)	74	7367 (320)	74
8 Months	0	0	219 (13)	23	3697 (56)	75	8005 (791)	80

Notes: [†]Percent initial recovery of NG from freshly amended SSL soil compared with nominal target concentration; subsequent recovery values show percent change from the initial analytically determined concentration in freshly amended soil during the 8-month study. Measured concentrations are based on USEPA Method 8330A; Values are means (n=3) and standard deviations (SD).

Table 6. Concentrations and Percent Recovery of CL-20 in Sassafras Sandy Loam Soil During the Eight-month Litter Decomposition Study with *Dactylis glomerata*

Nominal mg/kg	Measured initial mg/kg	1 Month % recovery	2 Months % recovery	3 Months % recovery	4 Months % recovery	6 Months % recovery	8 Months % recovery
0	BDL	BDL	BDL	BDL	BDL	BDL	BDL
100	108	97	84	85	88	84	70
500	479	100	81	101	113	104	106
1000	870	96	97	117	97	97	95
2500	2355	94	98	102	94	86	84
5000	4635	88	103	96	105	92	97
7500	7020	89	100	82	111	82	86
10000	10300	84	97	93	105	85	80
Mean	NA	93	94	97	102	90	88
SE	NA	2.2	3.2	4.4	3.5	3.0	4.5

Note: Measured concentrations (means and standard errors, SE; n=3) are based on USEPA Method 8330A; BDL, below detection limit (0.06 mg/kg). NA, not applicable.

3.2 Effects of EMs on Litter Decomposition

Exposure of *D. glomerata* straw to 2,4-DNT in SSL significantly (p=0.032) inhibited litter decomposition (greater percentage of mass remaining) in the 9343 mg/kg treatment after one month compared with carrier control (Figure 1). Inhibition in this treatment remained significant ($p \leq 0.001$) throughout the eight-month study. Litter decomposition was also significantly ($p \leq 0.003$) inhibited in the 1274 mg/kg treatment after six and eight months compared with carrier control (Figure 1), thus indirectly suggesting an adverse effect on microbial activity at these 2,4-DNT concentrations in soil. Decomposition was significantly ($p < 0.015$) stimulated in the 3.8 and 62 mg/kg treatments after four and six months compared with carrier control. However, this effect was transient and percent of mass remaining in each of these treatments was not significantly ($p \geq 0.507$) different from carrier control by the end of the eight-month study (Figure 1).

There was a transient stimulatory effect of 2-ADNT on litter decomposition after the four-month exposure (Figure 2). Litter decomposition was significantly ($p \leq 0.042$) increased in the ≥117 mg/kg 2-ADNT treatments compared with carrier control after two and four months, and remained significantly (p=0.001) greater in the 10,000 mg/kg treatment after six months (Figure 2). However, percent of mass remaining in any of 2-ADNT treatments was not statistically ($p \geq 0.279$) different compared with carrier control by the end of the eight-month study (Figure 2). Similarly, litter decomposition was not significantly ($p \geq 0.10$) different among any of the 4-ADNT treatments throughout the eight-month study (Figure 3).

Exposure to NG significantly ($p \leq 0.001$) inhibited litter decomposition in the ≥950 mg/kg NG treatments compared with carrier control during the eight-month study (Figure 4). Litter decomposition in the 29 mg/kg treatment was not significantly ($p \geq 0.134$) different from carrier control during the same exposure period (Figure 4).

There was no statistically significant inhibition of litter mass loss in any of the CL-20 treatments compared with carrier control, except for a short-term significant (p=0.001) inhibition in the 108 mg/kg CL-20 treatment after 4 months (Figure 5). Litter decomposition was actually significantly ($p < 0.05$) stimulated in several CL-20 treatments (mg/kg) including 7020 after 1 month, 870 after 2 months, and 2355 after 4 and 6 months. By the end of the 8-month CL-20 investigation, mass loss by Orchard grass litter was significantly ($p < 0.05$) greater in the 108, 870, and 7020 mg/kg CL-20 soil treatments compared with carrier control.

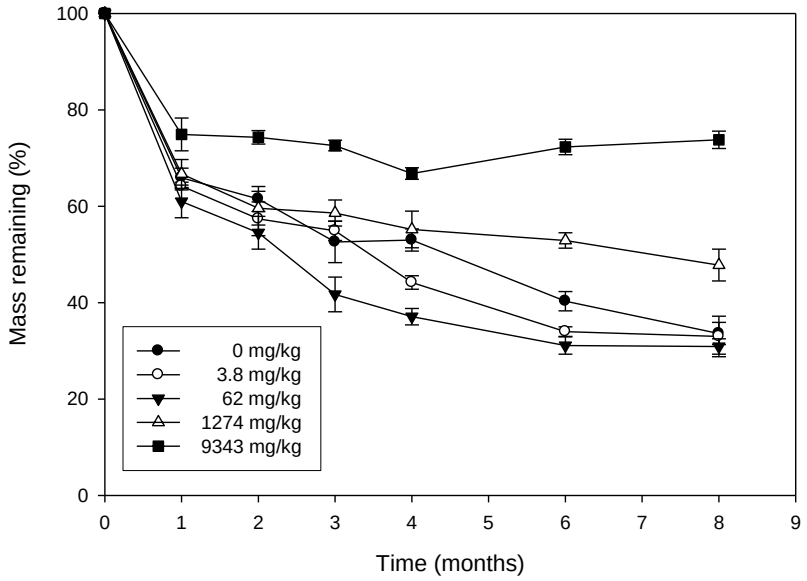

Figure 1. Effect of 2,4-DNT on decomposition of Orchard grass (*Dactylis glomerata)* litter in Sassafras sandy loam soil.
Note: Values are means ± SE (n=4).

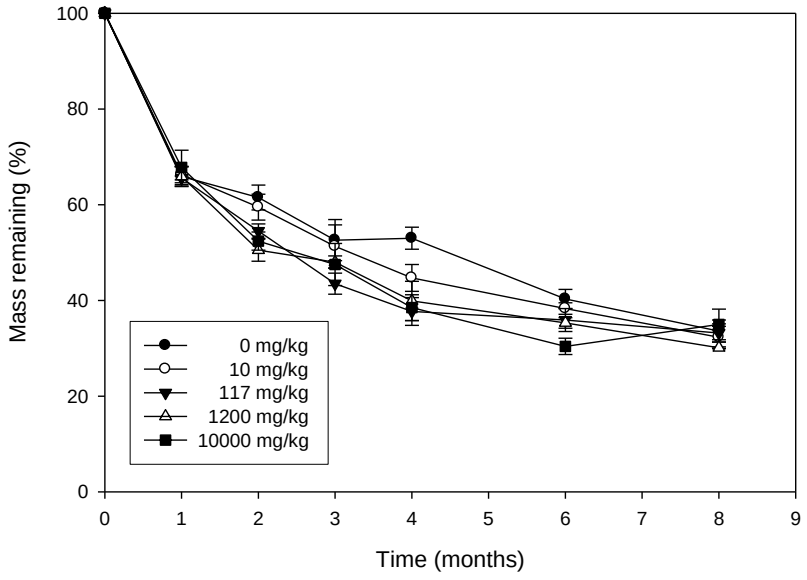

Figure 2. Effect of 2-ADNT on decomposition of Orchard grass (*Dactylis glomerata)* litter in Sassafras sandy loam soil.
Note: Values are means ± SE (n=4).

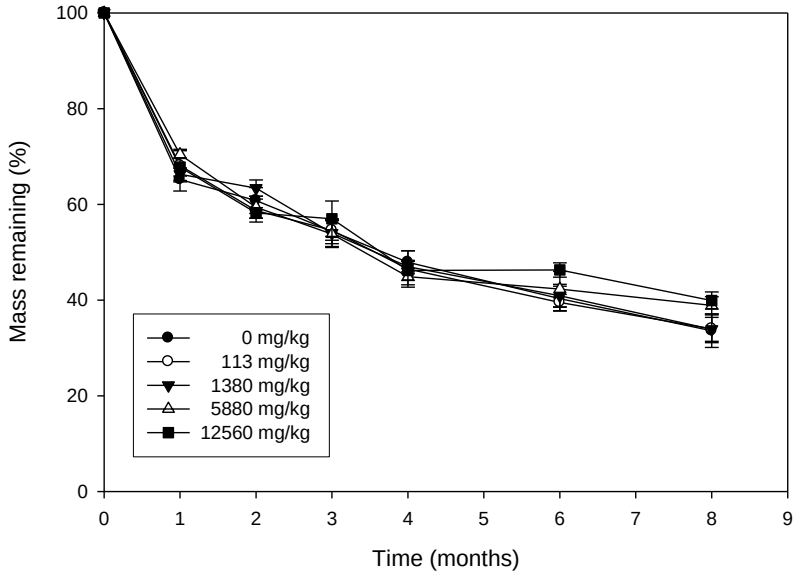

Figure 3. Effect of 4-ADNT on decomposition of Orchard grass (*Dactylis glomerata)* litter in Sassafras sandy loam soil.
Note: Values are means ± SE (n=4).

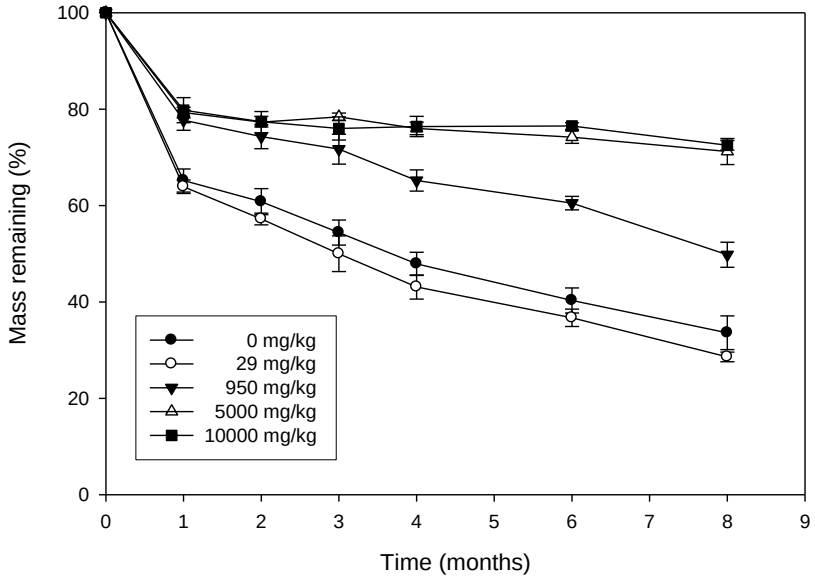

Figure 4. Effect of NG on decomposition of Orchard grass (*Dactylis glomerata)* litter in Sassafras sandy loam soil.
Note: Values are means ± SE (n=4).

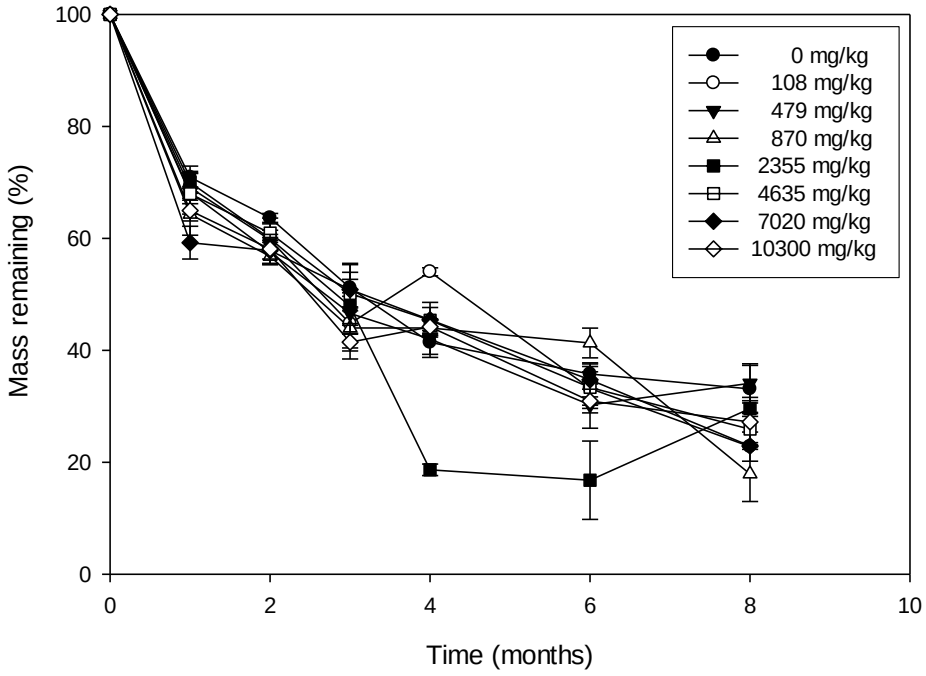

Figure 5. Effect of CL-20 on decomposition of Orchard grass (*Dactylis glomerata)* litter in Sassafras sandy loam soil.
Note: Values are means ± SE (n=4).

Annual decomposition rate constants (*k*), which integrate mass loss data over the entire study period, were significantly decreased (corresponding to slower rates of decomposition) in the two greatest 2,4-DNT treatments (1274 mg/kg and 9343 mg/kg), and in all positive NG treatments, except the lowest (29 mg/kg); all compared to respective carrier controls (Table 7). There were no significant effects on decomposition rate constants in any of 2-ADNT ($p \geq 0.153$) or 4-ADNT ($p \geq 0.065$) treatments (Table 8). Litter decomposition rate constants were statistically similar ($p > 0.05$) in all CL-20 treatments except in the nominal 2500 mg/kg CL-20, in which *k* value was significantly ($p = 0.029$) greater compared with carrier control or other CL-20 treatments tested (Table 9). The increase in decomposition rate in the 2500 mg/kg CL-20 treatment was due to a greater litter mass loss on the 4th and 6th month harvest dates (Figure 5).

The ranges of 2,4-DNT and NG concentrations selected for these studies were sufficient to establish the concentration-response relationships for the effects of respective EM on litter decomposition over the entire eight-month study (Figures 6A and 6B) based on annual decomposition rate constants (*k*) for litter residues shown in Table 7.

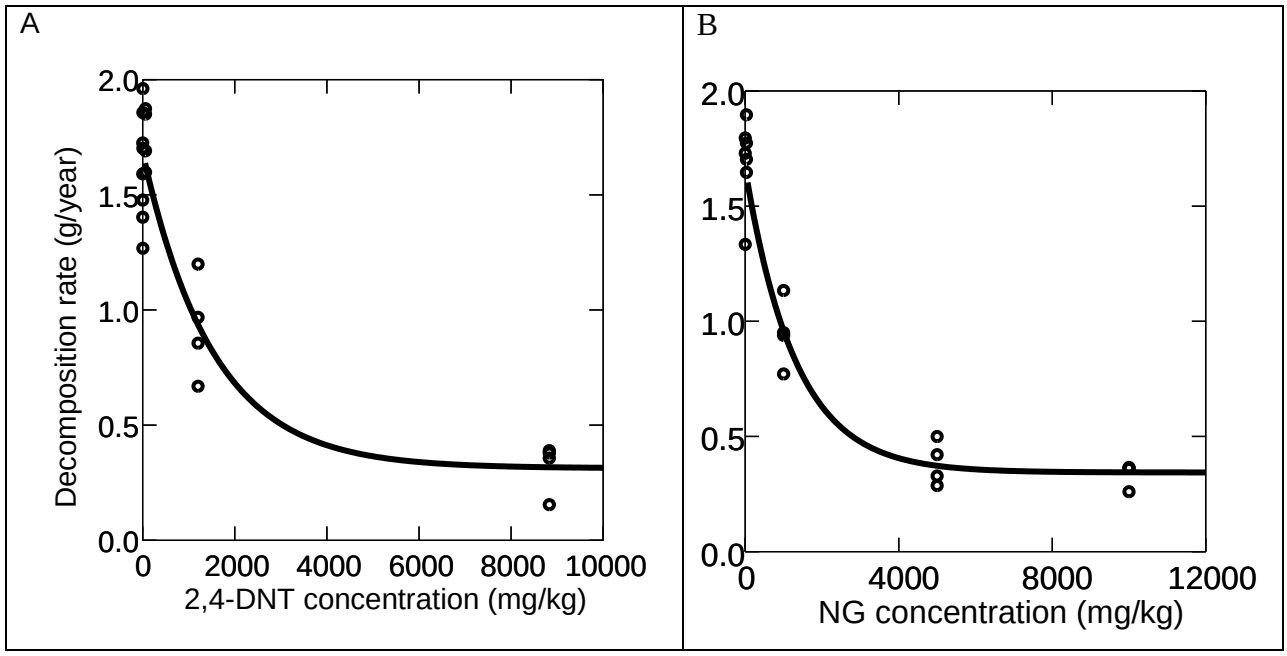

Figure 6. Effects of 2,4-DNT (A) and NG (B) on annual decomposition rate of Orchard grass (*Dactylis glomerata)* litter in Sassafras sandy loam soil.

Table 7. Decomposition Rate Parameters for Orchard Grass (*Dactylis glomerata)* Litter Exposed to 2,4-DNT or NG in SSL Soil for Eight Months

2,4-DNT† mg/kg	*k*	r^2	NG† mg/kg	*k*	r^2
0	–1.550 ± 0.22[a]	0.911	0	–1.547 ± 0.22[a]	0.908
3.8	–1.696 ± 0.26[a]	0.895	29	–1.755 ± 0.24[a]	0.913
62	–1.753 ± 0.38[a]	0.813	950	–0.948 ± 0.12[b]	0.919
1274	–0.922 ± 0.27[b]	0.698	5000	–0.383 ± 0.14[c]	0.590
9343	–0.320 ± 0.22[c]	0.298	10000	–0.337 ± 0.15[c]	0.496

Notes: †Values are soil concentrations analytically determined using USEPA Method 8330A; Annual decomposition rate constants (*k*) and coefficients of determination (r^2) are based on a single negative exponential model; *k* values are means ± SE (n=4); For each energetic material *k* values with the same letter are not significantly different (ANOVA and FLSD test at $p \leq 0.05$ level).

Table 8. Decomposition Rate Parameters for Orchard Grass (*Dactylis glomerata)* Litter Exposed to 2-ADNT or 4-ADNT in SSL Soil for Eight Months

2-ADNT† mg/kg	*k*	r^2	4-ADNT† mg/kg	*k*	r^2
0	–1.550 ± 0.22	0.911	0	–1.547 ± 0.22	0.908
10	–1.644 ± 0.24	0.906	113	–1.557 ± 0.23	0.902
117	–1.625 ± 0.37	0.797	1380	–1.546 ± 0.22	0.909
1200	–1.723 ± 0.31	0.859	5880	–1.366 ± 0.28	0.828
10000	–1.689 ± 0.39	0.790	12560	–1.238 ± 0.28	0.798

Notes: †Values are soil concentrations analytically determined using USEPA Method 8330A; Annual decomposition rate constants (*k*) and coefficients of determination (r^2) are based on a single negative exponential model; *k* values are means ± SE (n=4). Among treatments of each energetic material *k* values were not significantly different (ANOVA and FLSD test at $p \leq 0.05$ level).

Table 9. Decomposition Rate Parameters for Orchard Grass (*Dactylis glomerata)* Litter Exposed to CL-20 in SSL Soil for Eight Months

CL-20 mg/kg	*k*	SE	r^2
0	–1.616	0.236	0.904
100	–2.003	0.231	0.938
500	-1.620	0.312	0.843
1000	–2.108	0.340	0.885
2500	–2.312*	0.778	0.639
5000	–1.888	0.151	0.969
7500	–1.902	0.215	0.940
10000	–1.819	0.262	0.906

Notes: Nominal CL-20 concentrations are reported. Analytically determined initial concentrations of CL-20 in SSL soil and percent recovery after 1, 2, 3, 4, 6, and 8 months of incubation are shown in Table 6. *Significantly different (p=0.029) compared to other treatments.

Table 10. Toxicity Benchmarks for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 Effects on Decomposition of Orchard Grass (*Dactylis glomerata)* Litter in Sassafras Sandy Loam Soil

Ecotoxicological parameters	2,4-DNT[†] (mg/kg)	2-ADNT (mg/kg)	4-ADNT (mg/kg)	NG[†] (mg/kg)	CL-20 (mg/kg)
NOEC	62	10	12560[††]	29	10300[††]
p	0.161	0.073	0.280	0.064	
LOEC	1274	117[‡]	>12560	950	>10300
p	<0.0001	0.003		<0.0001	
EC_{20}	361	>10000	>12560	277	>10300
95% CI	168–554			158–395	
EC_{50}	1122	>10000	>12560	860	>10300
95% CI	523–1721			492–1228	
r^2	0.981	ND	ND	0.984	ND

Notes: Values are soil concentrations analytically determined using USEPA Method 8330A. EC, effect concentration; NOEC, no-observed-effect concentration; LOEC, lowest-observed-effect concentration. [†]Values determined on the basis of annual decomposition rate constants; [‡]A transient effect, based on ANOVA and FLSD test of percent mass remaining after a four-month exposure; No significant difference in mass loss ($p>0.05$) among any 2-ADNT concentrations at the end of the eight-month study (corresponding unbounded NOEC value is 10,000 mg/kg) [††]Unbounded NOEC based on ANOVA and FLSD test of percent mass remaining during the eight-month exposure; ND, not determined.

Nonlinear regression analyses of annual decomposition rate data yielded the EC_{20} and EC_{50} values (mg/kg) of 361 and 1122, respectively, for 2,4-DNT; and 277 and 860, respectively, for NG. Table 10 summarizes ecotoxicological benchmarks for the effects of 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 on litter decomposition.

4. DISCUSSION

Maintaining soil quality, fertility, and structure is essential for protecting and sustaining biodiversity and ecological integrity of terrestrial ecosystems. Central to achieving this goal is the need for a greatly improved understanding of the potential effects of EM contaminants on the sustainability of ecosystems at defense installations, and the development of environmental quality criteria that can be consistently applied in order to gauge the ecotoxicological impacts of the military operations. Litter decomposition is among the most integrating processes within the soil ecosystem because it involves complex interactions of soil microbial, plant, and faunal activities with the soil chemical environment. Any disturbance that alters this biologically-mediated process can result in nutrient losses and a decline in soil fertility, which can negatively impact sustainability of the environment. Furthermore, the process of litter decomposition is essential for sustainment of ecosystems. Therefore, an assessment of how soil contamination with EMs may alter rates of litter decomposition is critical to understanding their potential impacts on the overall functioning of the soil ecosystem at military testing and training sites.

Nitroaromatic energetic materials introduced into soil during testing and training activities at defense installations can undergo rapid transformation to the amino-nitro

intermediates (Kuperman et al., 2009b; Monteil-Rivera, 2009) resulting in frequent co-occurrence of DNTs, and ADNTs in soils of contaminated sites. This has precluded investigators from partitioning the effects of the parent materials and their transformation products on soil microorganisms (Fuller and Manning, 1998; Gong et al., 1999; 2000). Therefore, we designed our litter decomposition studies to investigate the effects of individual nitroaromatic EMs 2,4-DNT, 2-ADNT, 4-ADNT, as well as the effects of NG, and the nitramine CL-20.

The present studies have established new ecotoxicological data for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 effects on litter decomposition in a sandy loam soil, and these results denote what may be expected in other comparable coarse-textured soils. The results of these studies showed that soil contamination with 2,4-DNT or NG can inhibit litter decomposition rates while exposure to 2-ADNT, 4-ADNT or CL-20 did not significantly affect litter decomposition in SSL soil. These results are in agreement with data determined in the substrate-induced respiration (SIR) studies that utilized similar SSL soil. Those SIR studies established comparable EC_{20} and EC_{50} values of 878 mg/kg and 1446 mg/kg, respectively, for 2,4-DNT (Sunahara, 2012), and the no-observed-adverse-effect-concentration (NOAEC) values 6078 mg/kg for 2-ADNT and 7819 mg/kg for 4-ADNT (Sunahara, 2012). Akin to the effects on litter decomposition, NG inhibited the SIR in SSL soil within the range of NG concentrations selected for these studies, but the EC_{20} and EC_{50} values were an order of magnitude lower (25 mg/kg and 77 mg/kg, respectively), compared with the corresponding values for litter decomposition (Table 10). These results suggest that the SIR can be a more sensitive endpoint for assessing NG effects on biologically-mediated processes in soil, compared with litter decomposition.

The present studies showed indirectly that soil biotic activity that controls the rate of litter decomposition in SSL soil was either unaffected or stimulated by exposure to CL-20 in soils up to and including 10,300 mg/kg. This conclusion is also supported by the findings of the microcosm study (Kuperman et al., 2013) that assessed the effects of CL-20 on the microinvertebrate community in the same test units used in these litter decomposition assays. This microcosm study showed that overall composition of microarthropod community in SSL soil was not affected by exposure to CL-20, based on the number of taxonomic group present in the individual treatments after 12 weeks. The equally-represented oribatid mites and collembola were the second most abundant individual groups among microarthropods (prostigmatid mites were most abundant), and jointly comprised approximately 40% of the microarthropod community throughout the study. Grazing activity of the oribatid mites and collembola provide the greatest contribution to litter decomposition among studied groups of microarthropods. Furthermore, dominance of bacterivore and fungivore nematodes among the nematode community, and the increases in their absolute numbers that were sustained throughout the study, suggest indirectly that availabilities of their respective food sources (bacteria and fungi), were also unaffected-or-increasing in soil with CL-20 treatments. This is important because bacteria and fungi play the primary role in litter decomposition though production of extracellular enzymes involved in organic matter breakdown.

The direct evidence supporting the hypothesis that the soil microbial community can tolerate or be stimulated by exposure to CL-20 comes from the studies by Gong et al. (2004). Those authors demonstrated in definitive studies with similar SSL soil that indigenous soil

microorganism were unaffected by exposure to CL-20 at ≤10,000 mg/kg (Gong et al. 2004). Using dehydrogenase activity (DHA) and potential nitrification activity (PNA) assays, Gong et al. (2004) showed that DHA was actually stimulated in 100 mg/kg CL-20 treatment, PNA was stimulated in the 1000 mg/kg CL-20 treatment, and both were stimulated in the 10,000 mg/kg CL-20 treatment. Taken together, results of our study of Orchard grass litter decomposition and those reported by Gong et al. (2004) show that exposure to CL-20 at ≤10,000 mg/kg concentration does not adversely affect microbial activity in SSL soil. These results also show that litter decomposition and other microbial activity-based assessments can be less sensitive endpoints for determining the effects of CL-20 on soil organisms, compared with standardized single-species soil invertebrate toxicity tests, as demonstrated by CL-20 toxicity data for the potworm *Enchytraeus crypticus* (Kuperman et al. 2006b).

Overall, the results of our studies show that assessment of the soil microbial activity endpoints provide valuable information on the EM effects on critical ecosystem-level processes such as energy and nutrient cycling, and can complement and expand upon the ecotoxicological significance of data from the standardized single-species toxicity tests, thereby meeting DoD stewardship goals plus promoting management for sustainable use of military ranges.

5. CONCLUSIONS

Present studies were designed to develop scientifically defensible toxicity data, to establish information required for successful management of defense installations in a sustainable manner and for the knowledge-based decision making. These studies have established new ecotoxicological data for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 effects on litter decomposition in a sandy loam soil, and these results denote what may be expected in other comparable coarse-textured soils. The results showed that soil contamination with 2,4-DNT or NG can inhibit litter decomposition rates while exposure to 2-ADNT, 4-ADNT or CL-20 did not significantly affect litter decomposition in SSL soil.

The exposure concentrations of 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 in soil were analytically determined at the beginning of each definitive toxicity test; consequently, the ecotoxicological benchmarks were determined using measured EM concentrations. Chemical analyses of amended soils using U.S. EPA Method 8330A showed good correlation between nominal and measured concentrations, which confirmed that the soil amendment procedures used in the definitive toxicity assays were appropriate, and that the analytical methodology was efficient for quantifying the amounts of 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20 in soil.

Assessment and protection of the terrestrial environment at defense installations can be advanced by applying scientifically based ecotoxicological benchmarks developed in the present studies for 2,4-DNT, 2-ADNT, 4-ADNT, NG, and CL-20. These ecotoxicological benchmarks can help to identify concentrations of contaminant EM in soil that present an acceptable ecological risk for biologically-mediated processes in soil. Then managers may better focus remediation resources on those EMs that present unacceptable risk. Applying microcosm

design to ecotoxicological investigations can improve ecological risk assessment by incorporating ecological principles into ERA methodologies.

LITERATURE CITED

ASTM International. *Standard Specification for Reagent Water*; ASTM D1193-99. ASTM International: West Conshohocken, PA, 2004; UNCLASSIFIED Specification. http://www.astm.org/Standards/D1193.htm (accessed March 2013).

Environment Canada. *Guidance Document on Statistical Methods for Environmental Toxicity Tests;* EPS 1/RM/46; Environment Canada: Quebec, March 2005.

Fuller, M.E.; Manning, J.F. Evidence for differential effects of 2,4,6-trinitrotoluene and other munitions compounds on specific subpopulations of soil microbial communities, *Environ. Toxicol. Chem.*, **1998**, 17, pp 2185-2195.

Gong, P.; Sunahara, G.I.; Rocheleau, S.; Dodard, S.G.; Robidoux, P.-Y.; Hawari, J. Preliminary ecotoxicological characterization of a new energetic substance, CL-20. *Chemosphere* **2004**, 56, pp 653-658.

Gong P.; Gasparrini, P.; Rho, D.; Hawari, J.; Thiboutot, S.; Ampleman, G.; Sunahara, G.I. An in situ respirometric technique to measure pollution-induced microbial community tolerance in soils contaminated with 2,4,6-trinitrotoluene, *Ecotoxicol. Environ. Saf.* **2000**, 47, pp 96-103.

Gong, P.; Siciliano, S.D.; Greer, C.W.; Paquet, L.; Hawari, J.; Sunahara, G.I. Effects and bioavailability of 2,4,6-trinitrotoluene in spiked and field-contaminated soils to indigenous microorganisms, *Environ. Toxicol. Chem.* **1999**, 18, pp 2681-2688.

Hawari, J. Biodegradation of RDX and HMX: From basic research to field application. In: Spain, J.C., Hughes, J.B., Knackmuss, H. (Eds.), Biodegradation of Nitroaromatic Compounds and Explosives. Lewis Publishers, Boca Raton, FL, USA, 1999, pp. 277–310.

Hawari, J.; Deschamps, S.; Beaulieu, C.; Paquet, L.; Halasz, A. Initial reactions in the photodegradation of CL-20 in aqueous solution: insights into the mechanisms of initial reactions and environmental fate. *Water Res*., **2004**, *38*, pp 4055-4064.

Jenkins, T.F. *Energetic Munitions Constituents on DoD Training Ranges: Deposition, Accumulation, and Appropriate Characterization Technology*. Final Report. In: Strategic Environmental Research and Development Program (SERDP) and Environmental Security Technology Certification Program (ESTCP) Technical Exchange Meeting on DoD Operational Range Assessment and Management Approaches; October 2007; pp B-3–B-24; http://serdp-estcp.org/content/download/8237/101218/file/RAWorkshopRDTENeedsRpt.pdf (accessed March 2013).

Jenkins, T.F.; Hewitt, A.D.; Grant, C.L.; Thiboutot, S.; Ampleman, G.; Walsh, M.E.; Ranney, T.A.; Ramsey, C.A.; Palazzo, A.; Pennington, J.C. Identity and Distribution of Residues of Energetic Compounds at Army Live-Fire Training Ranges. *Chemosphere* **2006**, *63*, pp 1280–1290.

Kuperman, R.G.; Checkai, R.T.; Phillips, C.T.; Simini, M.; Anthony, J.S. *Effects of Nitramine Explosive CL-20 on the Soil Microinvertebrate Community in a Sandy Loam Soil*; ECBC-TR-1130, U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2013; UNCLASSIFIED Report.

Kuperman, R.G., Simini, M., Siciliano, S.; Gong, P. Effects of Energetic Materials on Soil Organisms. In: *Ecotoxicology of Explosives*; Sunahara, G.I., Lotufo, G., Kuperman, R.G., and Hawari, J., Eds; CRC Press: Boca Raton, FL, 2009a, pp 35–76.

Kuperman, R.G., Checkai, R.T., Johnson, M., Robidoux, P.Y., Lachance, B., Thiboutot, S.; Ampleman, G. Ecological Risk Assessment of Soil Contamination with Munition Constituents in North America. In: *Ecotoxicology of Explosives*; Sunahara, G.I., Lotufo, G., Kuperman, R.G., and Hawari, J., Eds; CRC Press: Boca Raton, FL, 2009b, pp 277–307.

Kuperman, R.G.; Checkai, R.T.; Simini, M. Environmental fate and transport of a new energetic material CL-20. Final Report, SERDP ER-1254. 2006a. http://www.serdp.org/content/download/5017/71714/file/ER-1254-FR.pdf (accessed March 2013).

Kuperman, R.G.; Checkai, R.T.; Simini, M.; Phillips, C.T.; Anthony, J.S.; Kolakowski, J.E.; Davis, E.A. Toxicity of Emerging Energetic Soil Contaminant CL-20 to Potworm *Enchytraeus crypticus* in Freshly Amended or Weathered and Aged Treatments. *Chemosphere* **2006b**, *62*, pp 1282–1293.

Kuperman, R.; Knacker, T.; Checkai, R.; and Edwards, C. Multispecies and multiprocess assays to assess the effects of chemicals on contaminated sites. In: *Environmental Analysis of Contaminated Sites* (Sunahara, G., Renoux, A., Thellen, C., Gaudet, C. and Pilon, A. eds.). John Wiley and Sons, Ltd., 2002, pp 45-60.

Kuperman, R. Litter Decomposition and Nutrient Dynamics in Oak-Hickory Forests along a Historic Gradient of Nitrogen and Sulfur Deposition. *Soil Biol. Biochem.* **1999**, 31 pp. 237-244.

Monteil-Rivera, F.; Halasz, A.; Groom, C.; Zhao, J.-S.; Thiboutot, S.; Ampleman, G. Fate and Transport of Explosives in the Environment: A Chemist's View. In: *Ecotoxicology of Explosives;* Sunahara, G.I., Lotufo, G., Kuperman, R.G., and Hawari, J., Eds; CRC Press: Boca Raton, FL, **2009**, pp 5–33.

Pal, B.C.; Ryon, M.G. Database Assessment of Pollution Control in the Military Explosives and Propellants Production Industry; ORNL-6202; Oak Ridge National Laboratory: Oak Ridge, TN, 1986.

Spanggord, R.J.; Mill, T.; Chou, T.W.; Smith, J.H.; Lee, S. Environmental Fate Studies on Certain Munitions Wastewater Constituents. Final Report, Phase II — Laboratory Studies. SRI Project Number LSU-7934, (ADA099256). Menlo Park, CA: SRI International; 1980. http://www.stormingmedia.us/65/6529/A652990.html. (accessed March 2013).

Sunahara, G.I. Development of Toxicity Benchmarks and Bioaccumulation Data for N-based Organic Explosives for Terrestrial Plants and Soil Invertebrates. Final Report, SERDP ER-1416. 2012. http://serdp-estcp.org/content/download/17859/197874/file/ER-1416-FR.pdf, (accessed March 2013).

Sunahara, G.I.; Lotufo, G.L.; Kuperman, R.G.; Hawari, J. Ecotoxicology of Explosives. Boca Raton, FL: CRC Press; 2009.

U.S. Environmental Protection Agency (USEPA). *Guidance for Developing Ecological Soil Screening Levels*; OSWER Directive 9285.7-55; U.S. EPA: Washington, DC, 2005. http://www.epa.gov/ecotox/ecossl/ (accessed March 2013).

U.S. Environmental Protection Agency (USEPA). Nitroaromatics and Nitramines by High Performance Liquid Chromatography (HPLC). Method 8330A, Revision 1, February, 2007. In: *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods*, Third Edition, Final Update IV; SW-846; Office of Solid Waste, U.S. EPA: Washington, DC, 2007. http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/8330a.pdf (accessed March 2013).

U.S. General Accounting Office (GAO). *Military Munitions: DOD Needs To Develop a Comprehensive Approach for Cleaning Up Contaminated Sites*; GAO-04-147; Report to the Honorable John D. Dingell, Ranking Minority Member, Committee on Energy and Commerce, House of Representatives; U.S. GAO: Washington, DC; December 2003. http://www.gao.gov/new.items/d04147.pdf (accessed March 2013).

Verscheuren, K. *Handbook of Environmental Data on Organic Chemicals*. 2nd ed. Van Nostrand Reinhold, New York, NY; 1983.

Wentsel, R.S.; Beyer, W.N.; Edwards, C.A.; Kapustka, L.A.; Kuperman, R.G. Effects of Contaminants on Soil Ecosystem Structure and Function. In: LANNO, R.L., (Ed.). Contaminated Soils: From Soil-Chemical Interactions to Ecosystem Management. SETAC Press, 2003, pp.117-159.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ACN	acetonitrile
2-ADNT	2-amino-4,6-dinitrotoluene
4-ADNT	4-amino-2,6-dinitrotoluene
1,3-DNB	1,3-dinitrobenzene
2,4-DNT	2,4-dinitrotoluene
APG	Aberdeen Proving Ground
ASTM	American Society for Testing and Materials
BDL	below detection limit
CAS	Chemical Abstracts Service
CEC	cation exchange capacity
CI	confidence interval
CL-20	2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexaazaisowurtzitane (China Lake compound 20)
DHA	dehydrogenase activity
DoD	U.S. Department of Defense
EC_{20}	concentration producing a 20% inhibition from control
EC_{50}	median effect concentration (producing a 50% inhibition from control)
EC	Environment Canada
ECBC	U.S. Army Edgewood Chemical Biological Center
Eco-SSL	ecological soil screening level
ECp	estimate of effect concentration for a specified percent effect
EM	energetic material
EPA	Environmental Protection Agency
ERA	ecological risk assessment
FLSD	Fisher's least-significant difference
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (high melting explosive)
HPLC	high-performance liquid chromatography
HPLC-UV	high-performance liquid chromatography - ultraviolet
k	annual decomposition rate constants
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol/water partition coefficient
LOD	limit of detection
LOEC	lowest-observed-effect-concentration
N	nitrogen
NA	not applicable
ND	not determined
NG	nitroglycerin
NO_2	nitrogen dioxide
NOAEC	no-observed-adverse-effect-concentration
NOEC	no-observed-effect concentration
NRCC	National Research Council of Canada, Montreal, QC
p	probability value

PAR	photosynthetically active radiation
PNA	potential nitrification activity
PTFE	polytetrafluoroethylene
r^2	regression coefficient
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine (royal demolition explosive)
RSD	relative standard deviation
SD	standard deviation
SE	standard error
SERDP	Strategic Environmental Research and Development Program
SIR	substrate-induced respiration
SLERA	screening level ecological risk assessment
S/N	signal/noise (ratio)
SSL	Sassafras sandy loam
t	time
TNT	2,4,6-trinitrotoluene
USEPA	U.S. Environmental Protection Agency
USGAO	U.S. Government Accounting Office
v	volume


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